WHOLE BODY EXPOSURES TO A PHOSPHORIC ACIDS AEROSOL: I. SPONTANEOUS ACTIVITY EFFECTS IN WILD RODENT AND AVIAN SPECIES

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*Two inhalation-chamber studies were conducted to assess acute (2-h out-of-chamber) and subacute (≤6 d postexposure) spontaneous activity effects of whole-body phosphoric acids aerosol exposure(s) in black-tailed prairie dogs (*Cynomys ludovicianus*) and rock doves (*Columba livia*). The aerosol was generated using a red phosphorus/butyl rubber (RP/BR) mixture under development as a military obscurant. Each study involved (1) 3 RP/BR target concentration groups [0.0 (controls), 1.0, and 4.0 mg/L], (2) 24 prairie dogs or rock doves (8/group), with gender included as a factor, (3) a successive 3-phase paradigm (2 d preexposure; 4 and 2 d of about 80 min/d exposures to RP/BR for prairie dogs and rock doves, respectively; and 6 d postexposure), and (4) infrared detection of the rodents'/birds' home-cage movements. In-chamber atmospheres were uniform and acceptable for all exposures; median aerosol mass concentrations ranged from 0.76 to 0.89 mg/L and 3.46 to 3.74 mg/L for the 1.0 and 4.0 mg/L groups, respectively, with median phosphoric acid (H_3PO_4) readings of between 67.2 and 74.3%; median particles were ≤0.85 μm. Mortality was negligible; no prairie dogs died, but 1 male rock dove died on d 3 postexposure to two 4.0 mg/L target concentrations of RP/BR aerosol. Group × session interactions were significant for the acute activity counts of both species. The acute mean ambulatory (e.g., walking) counts of prairie dogs and the acute mean ambulatory and horizontal (e.g., preening) counts of rock doves exposed to 4.0 mg/L RP/BR aerosol were relatively less than those of the other groups after the first 2 or 1 exposures, respectively. Nevertheless, acute session*

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means for all groups approximated or exceeded the 23 h/d activity measured during the pre- and postexposure phases—data indicating that chamber confinement caused a temporary, sharp increase in activity for both species irrespective of RP/BR aerosol concentrations. No RP/BR concentration-related, subacute shifts in the activity of the rodents/birds were observed.

INTRODUCTION

Numerous behavioral-physiological variables have been studied as indices of sublethal toxicosis in animals (Brown, 1988; Weiss and Laties, 1975). Activity measurements have long been used to assess changes in the irritability, exertion, and stamina of animals exposed to toxicants and atmospheric pollutants (Boche and Quilligan, 1960; Mautz et al., 1985; Stinson and Loosli, 1979). Two basic types of activity are generally recognized in these animal studies: locomotor and spontaneous. Locomotor activity refers to diverse measures of exertive exercise (e.g., running wheel, treadmill, maze running); spontaneous activity refers to measures of home-cage, nonexertive movements of animals (e.g., walking, grooming, scratching).

Atmospheric levels of acid aerosols and related compounds pose potential health hazards to humans (Environmental Protection Agency, 1989). To date, most toxicological studies of these effects have dealt with sulfuric acid (H_2SO_4), nitric acid (HNO_3), ammonium sulfate ($(NH_4)_2SO_4$), ammonium bisulfate (NH_4HSO_4), and sulfur dioxide (SO_2). Currently, a red phosphorus/butyl rubber (RP/BR) compound is under development as a military obscurant by the U.S. Army (Burton et al., 1982; Yon et al., 1983); detonation of RP/BR-filled grenades produces a white smoke consisting mainly of phosphoric (H_3PO_4) and polyphosphoric acids (e.g., $H_4P_2O_7$, $H_5P_3O_{10}$) (Brazell et al., 1984). Inhalation-chamber studies of animal exposure to this smoke offer comparative toxicological and human-health models of acid aerosol effects.

Diverse H_2SO_4 exposure concentrations (0.08–300 mg/L) and durations (0.3 h to continuous 52 wk) have been investigated in numerous species, including dog, donkey, guinea pig, monkey, mouse, rabbit, and rat (Environmental Protection Agency, 1989). Approximately 40 years ago, Treon et al. (1950) found that the "decreased order of sensitivity" to H_2SO_4 among test animals could be ordered as guinea pig, mouse, rat, and rabbit. High concentrations ($\geq$8.0 mg/L) and long exposures ($\geq$18 h) have consistently been associated with acute toxic effects in certain of these species (Environmental Protection Agency, 1989). Key clinical effects (injuries) linked with H_2SO_4 or H_2SO_4 plus contaminants include laryngeal or bronchial spasm, deep pulmonary damage, hemorrhage, and pulmonary edema (Cavender et al., 1977; Wolff et al., 1979). Impaired pulmonary function has been cited as a common end point (Amdur et al., 1978; Loscutoff et al., 1985), but a number of researchers also report

no pulmonary effects from H_2SO_4 exposures (Alarie et al., 1973; Lewkowski et al., 1979). Obviously, procedural and comparative factors account for some of the inconsistencies among studies.

Prior reports of RP/BR aerosol effects on activity are limited to observations by Preache for locomotor behavior in albino rats (Aranyi, 1984). Preache used a "figure-8 maze" with infrared detectors set to assess the movements of rats at eight points throughout the maze (Norton et al., 1975). Groups of rats were given 4–16, 2.25-h inhalation-chamber exposures to RP/BR aerosol concentrations between 0.75 and 1.2 mg/L. Separate 20-min activity sessions were conducted immediately after the final exposure and 14 d later. Locomotor activity in these groups of rats was elevated during both postexposure sessions relative to control rats. The most pronounced increases in maze movements were reported for rats given 4 wk of 4 consecutive 2.25-h/d exposures to 0.75, 1.0, and 1.2 mg/L aerosols.

This article describes separate inhalation chamber studies of RP/BR aerosol-induced behavioral effects in black-tailed prairie dogs and rock doves. Acute ($\leq$2 h out-of-chamber) and subacute ($\leq$6 d postexposure) measurements of spontaneous activity variables were obtained to delineate the potential effects associated with whole-body exposure(s) to this phosphoric acids aerosol. The null hypotheses stated: Irritation of mucosal tissue and skin (plus possible lesions/edema produced in bronchial or lung tissue) caused by H_3PO_4 during and following exposures to 1.0 and 4.0 mg/L target concentrations of RP/BR aerosol would produce neither acute nor subacute differences in the home-cage walking or grooming/preening movements of prairie dogs/doves relative to nonexposed (0.0 mg/L; filtered-air control) rodents/birds.

METHODS

Detailed procedures describing animal care, inhalation systems, and aerosol measurements are given in Shumake et al. (1989, 1992) and Sterner et al. (1988, 1989, 1991); a brief description of these follows.

Rodents/Birds

Conduct of the studies adhered to provisions of the "Guide for the Care and Use of Laboratory Animals" (Department of Health, Education and Welfare, 1978) and the *Code of Federal Regulations 9* (parts 1–3) concerning animal welfare (Department of Agriculture, 1985).

Prairie Dogs Twenty-four black-tailed prairie dogs (12 male, 12 female) were used (see Jones et al., 1983). The prairie dogs were captured at Buckley Air National Guard Base, Aurora, Colo., using a burrow-flooding technique (Elias et al., 1974). Seven (3 male, 4 female) and 17 (9 male, 8 female) prairie dogs, respectively, were selected randomly from

captures in February (n = 110) and December 1987 (n = 96)—animals captured about 19 and 9 mo prior to the study. Official state collection permits (87-0047 and 88-0047) were acquired prior to the captures. Prairie dogs were confined, but not used in any other research, prior to this study.

Upon arrival at the Research Center, prairie dogs were sexed and placed in separate male and female communal areas (5.7 × 3.45 × 3.6 m). These areas were located within an isolated brick building; wood shavings (approximately 4 cm deep) were dispersed over the floor. The animals were fed Purina Rabbit Checkers (Purina Mills, Inc., St. Louis, Mo.) ad libitum, with fresh cabbage provided 3 times/wk; water was available ad libitum from several 3-gal poultry founts. The holding facility was both temperature (T = 18 ± 5°C) and 12 : 12-h light : dark controlled (0600–1800 : 1800–0600 h). All animals were quarantined for 14 d. Following quarantine, the prairie dogs were held under these conditions until the time of the study. Each prairie dog was implanted with a subcutaneous transponder (Identification Devices, Inc., Boulder, Colo.) for identification (Fagerstone and Johns, 1987).

Rock Doves Twenty-four wild-caught rock doves (11 male, 13 female) were selected randomly from 222 birds (Goodwin, 1983). All doves (pigeons) were purchased from a local supplier; the supplier stated that the doves were captured in the Denver area using cannon nets (Grubb, 1988). The doves were received in 2 shipments dated January 1987 (n = 122) and April 1988 (n = 100); 17 (4 male, 13 female) and 7 (7 male) selected doves had been captive about 18 and 2 mo, respectively. Rock doves were confined, but not used in any other studies, prior to this research.

Upon delivery, the birds were examined and held (≤30 doves/cage) in wire-mesh outdoor aviaries (3.0 × 1.5 × 1.8 m). Ad libitum Purina Pigeon Checkers (Purina Mills, Inc., St. Louis, Mo.), cracked corn, grit, and water were provided. After several weeks in these aviaries, the doves were sexed and moved to an 11.5-m-diameter steel Butler building that was T (20 ± 2°C) and 12 : 12-h light : dark controlled (0600–1800 : 1800–0600 h) for a 14-d quarantine. The doves were held in 1 of 3 large wire-mesh aviaries (1.6 × 3.3 × 2.6 m; 2.0 × 6.6 × 2.6 m; 3.9 × 3.9 × 2.6 m). After quarantine, the rock doves continued to be held under these conditions until the study began.

Aerosol Exposure Systems

Two inhalation chamber systems were used to expose the prairie dogs and rock doves to either RP/BR aerosol or filtered air: RP/BR aerosol system and filtered-air system.

Figure 1 illustrates the RP/BR aerosol system. Filtered/humidified room air was directed to a custom glass pipe junction (burn chamber),

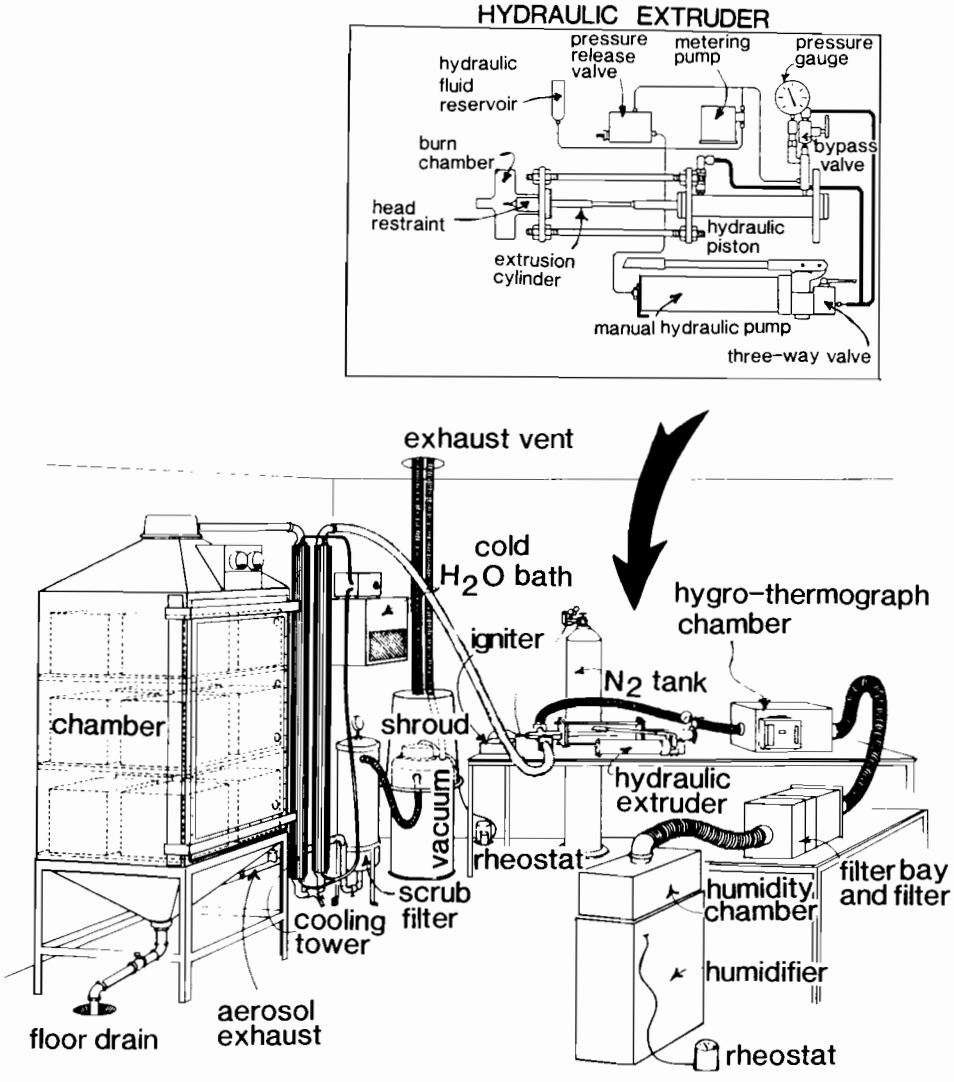

FIGURE 1. Illustration of the RP/BR aerosol system with a schematic of the RP/BR extruder shown in the insert. (Note: The scale is approximately 1 : 20. Components of the system are scaled relative to the perspective; the locations of certain components are drawn to improve the pictorial display.)

where RP/BR was extruded from a pipe cylinder under hydraulic pressure (300–1000 psi). The extruded bead (2 mm diameter) was ignited to generate RP/BR aerosol. As produced, aerosol was drawn through a U-shaped cooling tower (5 m) to the apex of the inhalation chamber. The inhalation chamber was a stainless steel unit (91.5 × 91.5 × 91.5 cm) with autoclave door (Bertke and Young, Cincinnati, Ohio). The chamber had 3 shelves each supporting 4 stainless steel wire mesh cages (30.5 × 30.5 ×

30.5 cm). A PVC drain valve was plumbed to the bottom of the chamber for flushing of residues.

From the apex of the chamber, the aerosol-laden air dispersed downward over the cages (laminar flow assumed). The rodent/bird exposure cage volumes were estimated at ≤ 0.10 of the available cage volume, and each rodent/bird was able to stand erect within the exposure cage. Aerosol exited the base of the chamber via polyvinyl chloride (PVC) pipe. A port for insertion of a wet-/dry-bulb thermometer to measure relative humidity (RH) was located in the first 30 cm of the exhaust line. From this RH port, aerosol moved to seven-bank, DX-grade coalescent filter unit (Balston Filter Products, Lexington, Mass.), which removed aerosol and associated contaminants from the chamber exhaust (Holmberg et al., 1985). Finally, the "scrubbed air" flowed to the vacuum source (Dayton Electrical Mfg., Co., Chicago, Ill.) via flexible polyethylene (PE) tubing, and exited the building through a ceiling vent. A 132-L PVC shroud, with exhaust hose connected to the ceiling vent, covered the vacuum source to prevent any residual aerosol products from entering the room.

For the production of RP/BR aerosol, a 95% RP/5% BR product was prepared by staff of the Bio-organic Analysis Section, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. After mixing of the dry RP and BR substances, the product was placed in a vacuum desiccator and hexane (C_6H_{14}) was introduced until 7–8% (w/w) was absorbed. This "softened product" was then loaded into 11.45-cm sections (1.91 cm ID) of stainless steel pipe (billets). Each billet contained approximately 40 g of pliable RP/BR material and was sealed with Teflon-lined steel caps to prevent drying. Only billets ≤ 3 mo old were used.

The filtered-air system was comprised of equipment and materials similar to the RP/BR-aerosol system; however, no cooling jacket or DX-grade filter unit was used with the filtered-air system because there was neither heat buildup nor aerosol present during the control exposures. The filtered-air system was used to expose separate groups of rodents/birds to filtered air for roughly equivalent time periods (0.0 mg/L controls); it was located in a separate room.

In-Chamber Atmospheric Measurements

Table 1 lists the atmospheric variables used to characterize in-chamber conditions of the RP/BR aerosol and filtered-air systems during exposure sessions with prairie dogs and rock doves; detailed descriptions of the sampling and analytical procedures are given in Sterner et al. (1988, 1989).

Table 2 presents summary statistics for the chamber atmospheric variables during the exposures of each species. Evaluations of the RP/BR aerosol measurements show that all groups of prairie dogs and rock doves were administered target concentrations of 1.0 and 4.0 mg/L of

TABLE 1. Variables and Respective Analytical Techniques Used to Characterize In-Chamber Conditions During the RP/BR Aerosol or Filtered-Air Exposures

Variable	Technique	Description
Aerosol mass	Gravimetric analysis	A 45-mm-diameter borosilicate-glass filter was positioned in a 1 L/min sample-flow line from the chamber to an air-flow pump; mass was measured gravimetrically using a digital analytical balance.
Phosphoric acid (H_3PO_4)	Titration analysis	Filters were stored in petri dishes for 48-168 h to allow for hydrolysis of acids. H_3PO_4 residue was extracted using 60 mL boiled deionized water during 10-min of stirring. A 20-mL sample of the solution was titrated with 0.1 *N* or 0.01 *N* sodium hydroxide (NaOH) using inflection-point analysis and a Radiometer DTS-800 multititration system (Radiometer America, Inc., Cleveland).
Aerosol opacity	ORNL Infrared detector	An infrared light-emitting diode mounted beside a phototransistor (Higgins et al., 1978; Holmberg et al., 1985) was inserted into the chamber; this provided an analog record (mV) of opacity on a chart recorder.
Aerosol particle sizes	QCM cascade impactor	Two 10-s (300-μl) samples of RP/BR aerosol were injected into a piezoelectric quartz-crystal-microbalance (QCM) cascade impactor (California Measurements Inc., Sierra Madre, Calif.). The MMAD for each sample was obtained using a graphical procedure (Chuan, 1986).
Respiratory gases		In-chamber O_2 and CO_2 levels were measured between 20 and 60 min of the exposure using the Gastec gas detection system (Gastec Inc., Newark, Calif.). Actual percent O_2 and ppm CO_2 were corrected for atmospheric pressure at 1646 m (5400 ft) elevation:
Oxygen (O_2)	Gastec tube (+31)	
Carbon dioxide (CO_2)	Gastec tube (2LL)	
		Corrected analyzer tube value = actual tube value × (760 mm Hg/628 mm Hg)
Contaminant gases		Measurements were made similar to those for O_2 and CO_2; however, CO, PH_3, and C_6H_{14} were reported as ppm data (corrected for 1646 m altitude).
Carbon monoxide (CO)	Gastec tube (1LL)	
Phosphine (PH_3)	Gastec tube (7L)	
Hexane (C_6H_{14})	Gastec tube (102L)	
Temperature/humidity		In-chamber *T* was recorded every 20 min using a digital thermometer. A wet-/dry-bulb thermometer was used to measure in-chamber RH near the end of each exposure; RH was determined using standard charts (Department of Commerce, 1965).
Temperature (*T*)	Digital thermometer	
Relative humidity (RH)	Wet-/dry-bulb thermometer	

TABLE 2. Median (Minimum–Maximum) Statistics for Selected Atmospheric Variables During the RP/BR Aerosol and Filtered-Air Chamber Exposures with Prairie Dogs and Rock Doves

	Prairie dogs target concentrations (extrusion setting)			Rock doves target concentrations (extrusion setting)		
Variable	0.0 mg/L	1.0 mg/L (50 μm)	4.0 mg/L (180 μm)	0.0 mg/L	1.0 mg/L (50 μm)	4.0 mg/L (180 μm)
Aerosol						
Aerosol mass (mg)	1.9 (−20.0–19.2)	62.3 (57.1–68.5)	290.2 (248.9–338.7)	3.85 (0.5–7.2)	71 (61.4–91.4)	299.5 (281.7–322.9)
Aerosol mass concentration (mg/L)	0.2 (0.00–0.19)	0.76 (0.4–0.86)	3.46 (1.50–4.03)	0.056 (0.01–0.12)	0.887 (0.77–1.14)	3.74 (3.52–4.04)
H_3PO_4 titration (mg)	ND	45.06 (40.06–49.82)	211.2 (83.5–238.9)	ND	46.91 (45.26–50.04)	217.6 (210.7–235.1)
H_3PO_4 concentration (mg/L)	ND	0.55 (0.37–0.62)	2.57 (1.04–2.78)	ND	0.586 (0.566–0.626)	2.72 (2.63–2.94)
Percent H_3PO_4 of aerosol mass	NM	73 (60–84)	73 (65–80)	NM	67.2 (51.6–73.7)	74.3 (69.7–73.7)
Particle size						
MMAD (μm)	NM	0.4 (0.4–0.4)	0.4 (0.4–0.8)	NM	0.4 (0.2–0.4)	0.6 (0.4–0.8)
Respiratory gases						
O_2 (%)	22	22 (21–23)	22 (21–22)	22 (21–22)	22 (18–22)	22 (20–22)
CO_2 (ppm)	847	696 (484–968)	847 (726–968)	605 (484–605)	605 (605–726)	787 (605–847)
CO (ppm)	ND	13 (10–22)	18 (15–21)	ND	12 (11–18)	20 (15–25)
PH_3 (ppm)	ND	ND	ND	ND	ND	ND
C_6H_{14} (ppm)	ND	ND	ND	ND	ND	ND
Exposure/chamber conditions						
Length of exposure (min)	86 (80–106)	80.5 (80–86)	81.5 (80–86)	85.5 (80–92)	80 (80–80)	80 (80–80)
Temperature (°C)	NA (21–25)	NA (20–24)	NA (21–24)	23 (21–26)	22.5 (21.5–24)	24 (23.5–26)
Relative humidity (%)	54 (50–63)	59.5 (52–67)	59 (48–82)	57.5 (52–62)	59 (52–66)	59 (52–63)

Note. NA, not available; ND, not detected; NM, not measured.

acceptable uniformity. Median aerosol mass concentrations for the actual exposures ranged between 0.76 and 0.89 mg/L and between 3.46 and 3.74 mg/L for the 4.0 mg/L target concentrations. Mass median aerodynamic diameter (MMAD) of aerosol particles was ≤ 0.85 μm—particle sizes typical of smokes. Median exposure durations ranged from 80.5 to 86 min for prairie dogs and from 80 to 85.5 min for rock doves. The RP/BR aerosol and filtered-air systems functioned well. There is every reason to conclude that the spontaneous activity effects observed are representative of prairie dogs and rock doves exposed to well-controlled, low-contaminant (no PH_3 or C_6H_{14}, with ≤ 24 ppm CO), oxygen-sufficient ($\geq 16\%$ O_2 and $\leq 1\%$ CO_2), respirable aerosol (MMAD $\leq$ 0.85 μm) and T-/RH-acceptable atmospheres (20–26°C; 48–82% RH).

Activity System

Eight Opto-varimex Activity Units (Columbus Instruments International Corp., Columbus, Ohio) composed the core of the activity system. Each unit consisted of a rectangular base (51.1 × 9.5 × 69.2 cm) with a large open area (43.2 × 44.4 cm) for insertion of a Plexiglas housing box (42.2 × 43.2 × 31.7 cm). It is estimated that rodent/bird housing box proportions were ≤ 0.05 of the available box volume, with all prairie dogs and rock doves able to stand erect within the boxes. Along 2 adjacent edges of the base were 15 small infrared sources (2.81 cm center-to-center distance); 15 infrared detectors were aligned along the opposite sides. The intersecting beams of 940-nm light formed a grid across the open area (housing box); interruptions of these "standing beams" by the animal/bird were tallied as spontaneous activity.

Two types of infrared-beam interruptions were recorded—horizontal and ambulatory. Horizontal breaks of the light beams generated 3-ms logic pulses each time that a beam in the 15 × 15 light matrix was interrupted; this count reflected all movements of a rodent/bird (e.g., licking, scratching, walking). Ambulatory breaks of the beams provided 3-ms logic pulses only after a "new" beam in the *x-y* axis was broken; thus, this count reflected only lateral movements of a rodent/bird (e.g., walking, jumping)—no output resulted from repeated interruptions of the same beam.

All hardware/software functions of the activity system were governed by a Horizon II Computer (Northstar Computers, Inc., Berkeley, Calif.), with activity counts automatically stored on floppy diskettes via a data scanner (Aeon Electronics, Denver, Colo.). A Hazeltine 1500 CRT terminal with keyboard (Hazeltine Corp., Greenlawn, N.Y.) provided the communications link with the system. Hard-copy printouts of daily activity files were obtained via a TI 820-RO printer (Texas Instruments, Inc., Houston, Tex.); automatic transmission of activity files to data analysis software was

accomplished via an AD-342 modem (Anderson-Jacobson, Inc., San Jose, Calif.).

Procedures

Separate studies were conducted with each species. These involved 3 groups each of 8 prairie dogs and 8 rock doves that received 4 and 2 successive approximately 80-min/d exposures (i.e., 20-min chamber fill, 40-min near target concentration, and 20-min chamber vent), respectively, to target concentrations of either 0.0 (filtered air), 1.0, or 4.0 mg/L RP/BR aerosol. Each study involved a 3-phase, sequential paradigm—a 2-d preexposure phase, a 4-d (prairie dogs) or 2-d (rock doves) exposure phase, and a 6-d postexposure phase. Gender was included as a factor in each study.

All inhalation-chamber exposures were conducted using the RP/BR aerosol and filtered-air systems. The activity measurements were conducted in a special temperature-controlled test room (2.7 × 3.7 × 2.8 m). Light was provided by 4 overhead fluorescent fixtures; a 12 : 12-h light-dark schedule (0600–1800, 1800–0600 h, respectively) was maintained throughout each study. The Opto-varimex units were the housing boxes for the rodents/birds throughout each study.

Prairie Dogs Following 5–10 d of acclimation, the 8 prairie dogs within each of 3 replications were rank ordered by body weight within gender class (4 males and 4 females per replication). The weight ranges of the males and females were 1109–1371 g and 958–1363 g, respectively, at the time of assignment. Animals were assigned quasi-randomly in sets of three or two (heaviest to lightest) to one of the three RP/BR aerosol groups. The term "quasi-randomly" refers to the constraints imposed on actual assignments within replications. Only eight activity units were used; hence, to balance the prairie dogs and sexes among the RP/BR aerosol groups, unequal numbers of rodents had to be assigned within groups for each replication. Specifically, replication 1 involved 2 (1 male, 1 female), 3 (1 male, 2 female), and 3 (2 male, 1 female) prairie dogs assigned to the 0.0, 1.0, and 4.0 mg/L RP/BR aerosol groups, respectively. Replication 2 involved 3 (2 male, 1 female), 2 (1 male, 1 female), and 3 (1 male, 2 female) rodents assigned to the 0.0, 1.0, and 4.0 mg/L groups, respectively. Replication 3 involved 3 (1 male, 2 female), 3 (2 male, 1 female), and 2 (1 male, 1 female) rodents assigned to the 0.0, 1.0, and 4.0 mg/L RP/BR aerosol groups, respectively. Modal (minimum–maximum) *T* and RH of the test room during the prairie dog study were 20°C (19–24) and 33% RH (21–88), respectively.

Throughout the three phases of the study, ad libitum Purina Rabbit Checkers (Purina Mills, Inc., St. Louis, Mo.) was provided in a

semicircular-shaped cup (6.5 cm diameter × 5 cm depth) located inside each housing cage above the infrared sensors. Ad libitum water was provided in two plastic bottles (200 and 100 mL graduated in mL) with rodent-lick spouts. Daily measurements of horizontal/ambulatory activity (infrared-beam interruptions), food intake (mg), water intake (mL), and body weight (g) were obtained; measurements reflected 23-h periods (0900–0800) and were obtained during a daily 1-h maintenance period (0800–0900 h).

On the day of exposure, handling procedures of the rodents differed slightly. Following the described maintenance procedures (0800–0859 h), exposures occurred at approximately 0900, 1130, and 1430 h for the 1.0, 4.0, and 0.0 mg/L groups, respectively. Consistent exposure schedules were used to (1) provide for increasing concentrations throughout the sequence (i.e., ease of flushing the chamber after a low concentration burn) and (2) determine the longest of the exposure durations for use with the daily filtered-air control group. Rodents were removed from their housing boxes, weighed, and transported to the inhalation test areas. Identifications were verified and each prairie dog was placed into a randomly designated exposure cage in the RP/BR aerosol or filtered-air chamber. After the animals were loaded into the chamber, the door was closed, and the approximately 80-min exposure was conducted (see Table 2). Upon completion of the exposure session, rodents were removed, reidentified, reweighed, and returned to their activity cages. After 2 h had elapsed, the horizontal/ambulatory activity counts were noted for each animal (acute measurements). Typically, prairie dogs in the 1.0, 4.0, and 0.0 mg/L RP/BR aerosol groups were out of their activity boxes at 0830–1100, 1130–1400, and 1430–1700 h, respectively, on the exposure days.

Rock Doves Procedures were basically the same as those described for prairie dogs. Main exceptions were (1) modal (minimum–maximum) *T* and RH for the activity room were 21°C (16–23.5) and 50% RH (34–90), respectively; (2) doves were fed Purina Pigeon Checkers (Purina Mills, Inc., St. Louis, Mo.) ad libitum; and (3) water was available ad libitum, but was provided in only one 100-mL graduated (mL) drinking tube, with an open reservoir protruding into each cage.

The 24 rock doves were acclimatized to housing conditions and were assigned to groups as were prairie dogs. Weight ranges of birds at the time of assignments were 257–387 g for males and 293–354 g for females. Specific assignments to 0.0, 1.0, and 4.0 mg/L groups were: replication 1, 2 (1 male, 1 female), 3 (1 male, 2 female), and 3 (2 male, 1 female) doves; replication 2, 3 (2 male, 1 female), 2 (1 male, 1 female), and 3 (0 male, 3 female); and replication 3, 3 (1 male, 2 female), 3 (2 male, 1 female), and 2 (1 male, 1 female).

Data Analyses

Acute (2 h out-of-chamber) ambulatory and horizontal activity counts for prairie dogs were analyzed as 3 (group) × 2 (gender) × 4 (session) factorials, with session treated as a repeated measures factor (Winer, 1971). Data for the acute counts in rock doves were analyzed as 3 (group) × 2 (gender) × 2 (session) factorials, with session treated as a repeated measures factor (Winer, 1971).

Subacute activity data (23-h horizontal and ambulatory counts) in both species were analyzed as 3 (group) × 2 (gender) × 8 (day) × 2 (lights on/off) factorials, where day and lights on/off factors were considered repeated measures factors (Winer, 1971). No exposure phase measurements were included in these analyses of variance (ANOVAs).

The sex of prairie dogs was balanced among groups (4 male, 4 female/group), but the gender of rock doves was unbalanced among groups (i.e., 4 male, 4 female for the 0.0 and 1.0 mg/L groups and 3 male, 5 female for the 4.0 mg/L group) because of the missexing of 1 bird using a cloacal examination technique (Miller and Wagner, 1955). This error was discovered during poststudy necropsy. Designs with balanced and no missing data were analyzed using PROC ANOVA (SAS Institute, Inc., 1985); designs involving unbalanced or missing data were analyzed using the general linear model and PROC GLM (SAS Institute, Inc., 1985). The means for significant terms obtained in respective ANOVAs were further analyzed using post hoc Duncan multiple range tests with $\alpha = 0.05$ (Duncan, 1955).

RESULTS

Table 3 lists the significant ANOVA interaction and main effects for the acute and subacute analyses computed for the horizontal and ambulatory activity counts of prairie dogs and rock doves.

Black-Tailed Prairie Dogs

No mortality occurred for this species during the conduct of the study. Shumake et al. (1992) gave detailed descriptions of pharmacotoxic signs and mortality ratios associated with several RP/BR aerosol exposure regimens for prairie dogs.

Acute Activity Effects No effects were found for acute horizontal activity as a result of RP/BR aerosol exposures in prairie dogs.

A significant group × session interaction ($F = 2.26$, df = 6/54, $p \leq .05$) occurred for the ambulatory activity counts in prairie dogs. Figure 2 is a bar graph of this interaction. Post hoc Duncan range tests revealed (1) mean counts for session 1 and session 2 of the 4.0 mg/L RP/BR aerosol group were significantly less than all other means, (2) means for session 3 of the 0.0 mg/L group were significantly greater than all other means, and

TABLE 3. Significant ANOVA Interaction and Main Effects for the Acute and Subacute Activity Analyses with Prairie Dogs and Rock Doves

		Horizontal activity				Ambulatory activity			
Species	ANOVA	Source	df[a]	*F*	*p*	Source	df[a]	*F*	*p*
Prairie dog	Acute					Group × session	6/54	2.26	≥ .05
	Subacute	Lights on/off	1/116	36.16	≥ .001	Lights on/off	1/116	30.12	≥ .001
Rock dove	Acute	Group × session	2/18	3.98	≥ .01	Group × session	2/17	4.51	≥ .026
		Session	1/18	6.63	≥ .01	Session	1/18	7.02	≥ .017
	Subacute	Gender × day × lights on/off	7/124	2.34	≥ .03	Gender × day × lights on/off	7/117	2.12	≥ .05
		Gender × day	7/124	2.07	≥ .05				
		Lights on/off	1/18	150.81	≥ .001	Lights on/off	1/17	680.71	≥ .001

[a]Certain df differed between the horizontal and ambulatory ANOVAs using PROC GLM due to specific missing data elements.

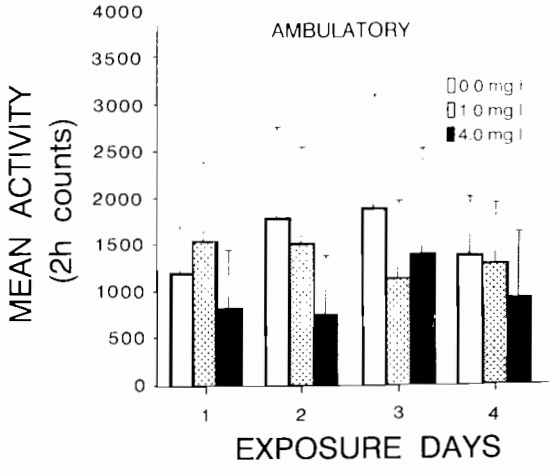

FIGURE 2. Bar graph of the mean (+SD) ambulatory activity counts for prairie dogs in the 0.0 (filtered-air control), 1.0, and 4.0 mg/L groups (n = 8/group) during the 2 h immediately after chamber confinement on each of the 4 exposure days—acute group × session interaction.

(3) means for session 2 of the 4.0 mg/L and session 4 of the 0.0 mg/L groups were less than and greater than all other means, respectively. Thus, this interaction is attributed to the disparity between the relatively lower counts for the 4.0 mg/L-exposed animals following the first 2 exposures and the elevated (nearly asymptotic) counts of the controls (0.0 mg/L) following the last 2 exposures. Interpretation of this mean activity pattern requires caution. Ambulation of all rodents actually increased compared to pre- and postexposure (see Subacute Activity Effects). The 2-h out-of-chamber ambulatory means of the groups (counts between 0800 and 1800 h) approximated or exceeded the average 23-h/d counts observed for the pre-/postexposure days.

Subacute Activity Effects Results of the ANOVAs for mean daily (23 h) activity on pre- and postexposure days in prairie dogs yielded only lights on/off main effects for the horizontal (F = 36.16, df = 1/116, $p \leq .001$) and ambulatory activity variables (F = 30.12, df = 1/116 $p \leq .001$). Mean (±SD) horizontal counts were 774.2 (±94.9) versus 395.7 (±39.1) and mean ambulatory counts were 387.2 (±28.1) versus 190.6 (±11.1) for the "on" versus "off" portions of the light cycle, respectively. For a diurnal species such as the black-tailed prairie dog (Jones et al., 1983), this pattern of means validates the Opto-varimex measurements and confirms that no subacute effects of RP/BR aerosol exposure upon spontaneous activity occurred.

Rock Doves

One male rock dove was found dead in its activity cage on the morning of d 4 postexposure of replication 3; this dove was in the 4.0 mg/L

group. Minimal overt signs preceded the death; the dove showed "listing-forward posture" and "unkempt feathers" within 24 h prior to death. Shumake et al. (1992) provided detailed descriptions of pharmacotoxic sign and mortality effects in rock doves exposed to selected RP/BR aerosol exposure regimens.

Acute Activity Effects Mean horizontal and ambulatory activity counts for the 2-h out-of-chamber sessions yielded significant group × session interactions (horizontal activity, $F = 3.98$, df = 2/18, $p \leq .01$, and ambulatory activity, $F = 4.51$, df = 2/17, $p \leq .026$) and session main effects (horizontal activity, $F = 6.63$, df = 1/18, $p \leq .01$, and ambulatory activity, $F = 7.02$, df = 1/18, $p \leq .017$). Post hoc Duncan tests for mean horizontal counts (preening) indicated that doves in the 4.0 mg/L aerosol group made significantly fewer movements immediately following the first exposure than doves in any of the other group × session cells of the design. These Duncan tests for mean ambulatory counts (walking) revealed that (1) doves exposed to 4.0 mg/L aerosol made fewer mean out-of-chamber walking movements than all other doves and (2) doves in the 0.0 mg/L group made greater mean walking movements immediately after exposure than doves in the RP/BR groups, with the mean activity for session 2 significantly higher relative to session 1.

This pattern of mean differences is somewhat similar to that described for prairie dog ambulatory activity. The initial exposure of doves to the 4.0 mg/L target concentration of aerosol caused relatively less activity than that of the 0.0 and 1.0 mg/L groups immediately after exposure, but a "chamber confinement effect" was observed for all groups of birds. The 2-h out-of-chamber counts for the 0.0 and 1.0 mg/L groups were elevated approximately six- to eightfold for horizontal activity and about two- to threefold for ambulatory activity relative to those in the 4.0 mg/L condition. Also, mean session counts greatly exceeded typical 23-h/d (subacute) activity. Figure 3 *a* and *b* are bar graphs of these acute (exposure day) horizontal and ambulatory activity interactions.

The session main effects also showed that mean horizontal and ambulatory activity counts were lower for session 1 than for session 2—data in agreement with the group × session interactions. Mean (±SD) horizontal-type counts for the acute sessions were 6221 (±3150) and 7666 (±3189) for session 1 and session 2, respectively; mean (±SD) 2-h out-of-chamber ambulatory counts were 3439 (±1993) and 4251 (±2101) for these respective sessions.

Subacute Activity Effects No RP/BR aerosol group (concentration-related) terms of the ANOVAs were significant for pre-/postexposure daily activity.

The ANOVAs for subacute activity of doves yielded a complex set of non-RP/BR-aerosol-induced results. The gender × day × lights on/off ($F = 2.34$, df = 7/124, $p \leq .03$) and gender × day interactions ($F = 2.07$, df = 7/124, $p \leq .05$), plus the lights on/off main effect ($F = 150.81$, df =

1/18, $p \leq .001$), were significant sources of variance for horizontal-type activity; the gender × day × lights on/off interaction ($F = 2.12$, df = 7/117, $p \leq .05$) and lights on/off main effect ($F = 680.71$, df = 1/17, $p \leq .001$) were significant for ambulatory activity.

The gender × day × lights on/off interactions for both horizontal and ambulatory activity are described jointly. Male and female rock doves differed in their daily diurnal and nocturnal home-cage activity, but these effects appeared to be induced by generalized chamber-confinement stressors during the exposure phase that affected the females more than the males (i.e., all groups showed this effect, including the control group). Post hoc Duncan tests indicated that (1) for the first postexposure day, mean "lights-on" (diurnal) activity counts of female doves were significantly less than for all other diurnal cell means; (2) the "lights-on" preexposure activity of female doves was greater than their diurnal postexposure activity (i.e., a reversal of activity for the female birds prior to and after the exposures); and (3) all diurnal versus nocturnal cell comparisons were significantly different from each other (i.e., lights on/off main effect). Figure 4 *a* and *b* illustrates the gender × day × lights on/off interactions for mean 23-h horizontal and ambulatory counts in the rock doves.

Similar to results for prairie dogs, the lights on/off main effects for both horizontal and ambulatory activity of the rock doves validated the Opto-varimex measurements. Rock doves are a diurnal species (Goodwin, 1983). Mean (±SD) horizontal counts were 2290.9 (±463.6) versus 923.5 (±101.2) and mean (±SD) ambulatory counts were 1298.8

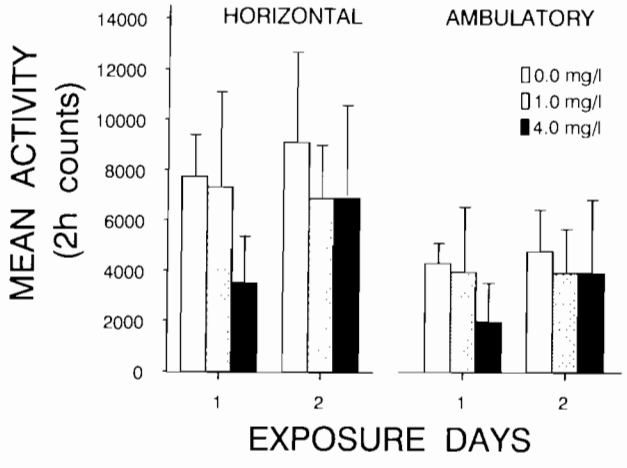

FIGURE 3. Bar graphs of the mean (+SD) (*a*) horizontal and (*b*) ambulatory activity counts for rock doves in the 0.0 (filtered-air control), 1.0, and 4.0 mg/L groups (n = 8/group) during the 2 h immediately after chamber confinement on each of the 2 exposure days—acute group × session interactions.

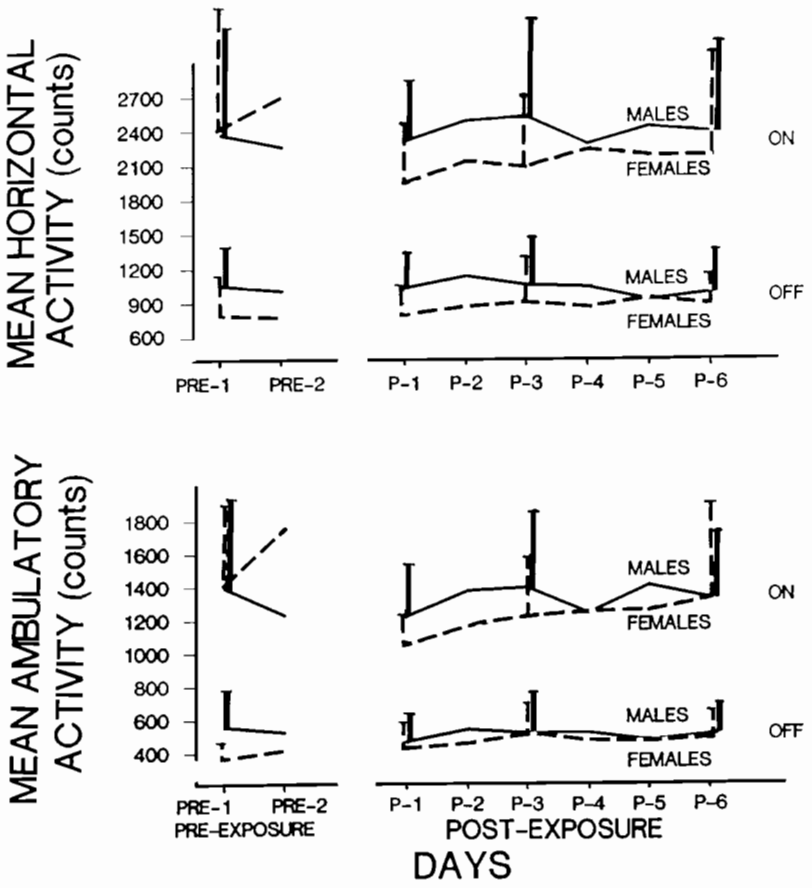

FIGURE 4. Line graphs of the mean (selected + SD) daily (11 h light on and 12 h light off) horizontal (top) and ambulatory activity counts (bottom) for male and female rock doves across the 8 d (pre-/postexposure) of the study—the subacute gender × day × lights on/off interaction.

(±672.6) versus 467.2 (±263.4) for light on versus off periods across the pre- and postexposure phases, respectively.

DISCUSSION

Some null hypotheses were rejected. Results show that 2 and 1 daily exposures to 4.0 mg/L target concentrations of RP/BR aerosol cause 2-h out-of-chamber (acute), relative decreases in the ambulatory activity of black-tailed prairie dogs and in the horizontal and ambulatory activity of rock doves; however, the activity of all groups of rodents/birds was elevated immediately after exposure. No subacute, RP/BR-aerosol-induced effects upon the home-cage activity of either species were observed.

Prairie dogs and rock doves in the 4.0 mg/L group decreased their ambulatory activity and ambulatory/horizontal activity between 30 and 50%, respectively, relative to the 0.0 and 1.0 mg/L groups immediately after the initial exposure sessions, whereas the 0.0 mg/L rodents/birds increased acute activity relative to the RP/BR aerosol groups after the later exposures. Because acute and subacute activity data were analyzed separately, the impacts of RP/BR aerosol exposure and chamber confinement were somewhat obscured. An acute chamber-confinement effect seemed to occur for all groups during the exposure phase—the mean 2-h out-of-chamber activity counts equaled or exceeded the pre-/postexposure day averages (i.e., 23-h/d means). In short, the rate of activity was increased immediately after chamber confinement. This result agrees with an earlier report by Preache for maze running in albino rats (Aranyi, 1984) but contrasts with that given by Sterner et al. (1989) in which the relative differences among the 0.0, 1.0, and 4.0 mg/L groups were emphasized.

The absence of significant effects for the 2-h out-of-chamber horizontal counts of prairie dogs implies that four 80-min/d exposures to 1.0 and 4.0 mg/L RP/BR aerosol concentrations had no immediate effects upon grooming/shaking/scratching activity in this species. The alternative hypothesis, that the potential irritation caused by H_3PO_4 on their fur/skin would lead to increased horizontal movements immediately after exposure, is unsupported. This could be indicative of the protection afforded by the rodents' fur, even during high-concentration exposures. Many prairie dogs had their heads tucked tightly into their bodies at completion of exposures. Of course, continued postexposure grooming allows for direct toxicological impacts via ingestion, a problem with all whole-body exposure studies.

The lack of H_3PO_4-related subacute activity effects in either prairie dogs or rock doves suggests that both species may be tolerant of the compound. Subacute activity effects in prairie dogs were limited to circadian differences, with the animals most active under the lights-on condition. Subacute effects in doves involved circadian and gender-related differences in walking or grooming/preening across pre- and postexposure days. This absence of aerosol-induced effects is contrary to the earlier report by Preache (Aranyi, 1984), which noted increased locomotor activity in albino rats both immediately and 14 d after RP/BR aerosol exposures. The current interactions suggest that exposure to RP/BR aerosol (4.0 mg/L) causes no subacute changes in activity for either prairie dogs or rock doves, but male and female doves react differently to chamber confinement regimen—effects that remain evident for several days.

Similarities in the toxicological and physiological effects observed for RP/BR aerosol (H_3PO_4 plus contaminants) and those for H_2SO_4 are striking (Johns et al., 1992; Shumake et al., 1989, 1992; Sterner et al., 1989). Obviously, morphological changes to the respiratory tract and deep pulmo-

nary function are affected end points. Shumake et al. (1989, 1992) cited "lost or affected vocalizations" as a key pharmacotoxic sign in both prairie dogs and rock doves exposed to ≥3.0 mg/L. The seemingly high concentrations (≥4.0 mg/L) and multiple exposures (four or two, 80 min/d) needed to produce even subtle pharmacotoxic or acute behavioral effects in prairie dogs and rock doves differ somewhat from earlier studies with albino rats; however, Burton et al. (1982) noted that five daily 60-min exposures of this species to RP/BR aerosol concentrations ≥5.0 mg/L caused laryngeal/epiglottal injuries. Still, current high dosages agree with a number of comparative observations described for H_2SO_4 and larger mammalian species (Environmental Protection Agency, 1989). To my knowledge, these assessments for rock doves are the only acid aerosol data available for a bird species.

The present mortality data for prairie dogs and rock doves concur with findings of Shumake et al. (1989, 1992). Shumake et al. (1989, 1992) reported greater tolerances of prairie dogs versus doves and greater mortality of male versus female doves (42 and 6%, respectively) to RP/BR aerosol—a finding supported by the death of one male rock dove in this study. Concentration effects associated with mortality in these species contrast markedly with prior reports for albino rats; roughly a fourfold lower RP/BR aerosol concentration (i.e., 0.75–1.25 mg/L) has been reported to induce mortality in rats (Aranyi, 1984; Aranyi et al., 1988; Burton et al., 1982).

In conclusion, the results offer no inferences to chronic studies. It is feasible that acid-induced irritations, lesions, and edema could take longer than 6 d to develop. Prior research has shown that the spontaneous activity of animals is altered by administration of specific drugs, contaminants, and hormones (Alfano and Petit, 1981; Segal et al., 1971). Circulating metabolites from uptake of these agents/substances are viewed to inhibit or stimulate "cortical arousal" via complicated inhibitory and excitatory projections of the ascending reticular activating system (ARAS) (Grossman, 1967)—a system of diffuse cortical pathways projecting from the reticular formation of the brainstem anterior through hypothalamic and thalamic areas to various cortical sites. The ARAS affects sleep–wakefulness, attention–inattention, and hypo–hyperactivity patterns of animals (French, 1960; Lindsley, 1960). The mechanism(s) by which ARAS excitation or inhibition could produce the current pattern of results is unclear. Predictions of increases (irritability/agitation) or decreases (lethargy/malaise) in animal/bird activity following exposure to different acid aerosol regimen remain viable hypotheses for acute, subacute, and chronic studies. Removal of rodents/birds from the reduced stimulation of the darkened, sealed (250 L/min ventilation) inhalation chambers to the lighted, open (9 air exchanges/h) test rooms could arouse/stimulate central nervous system (ARAS) evoked potentials immediately postconfinement. Similarly, tissue irritation and pulmonary function changes

(e.g., decreased respiratory frequency, increased residual volume, decreased dynamic compliance) induced by acid aerosol exposure could dampen/depress central nervous system evoked potentials long after exposure. Studies incorporating evoked cortical potential recordings at sites in the ARAS and cerebellum (grooming) would seem beneficial to delineate specific behavioral consequences of acid aerosol exposures.

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